

# A single sorbent for tetracycline enrichment and subsequent solid-matrix time-resolved luminescence<sup>☆</sup>

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## Abstract

The aim of this study was to search for a sorbent that could act as an extraction phase and as a support for solid-matrix time-resolved luminescence (SMTRL). Four potential sorbents were investigated for this purpose using tetracycline (TC) as a model analyte. Sorbents prepared from C18 silica gel or calcium cross-linked pectin gel were able to extract TC from dilute solutions. Europium(III)–TC complex adsorbed on the surface of C18 generated the most intense TRL signal when measured at  $\lambda_{\text{ex}} = 388 \text{ nm}$  and  $\lambda_{\text{em}} = 615 \text{ nm}$ . This method achieved a 1 ng/ml limit of detection (LOD) with a 100  $\mu\text{l}$  sample solution in a repeated spotting mode. Hyphenation of sorbent extraction and SMTRL was demonstrated using C18. This method is suitable for screening of TC in foods or aqueous solutions and can be extended to other luminescent lanthanide-chelating analytes in physiological or environmental samples.

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## 1. Introduction

Tetracycline (TC, Fig. 1) is a broad-spectrum antibiotic, used in farm animals for the treatment of a wide range of bacterial infections. Although TC is generally regarded as relatively non-toxic, frequent use results in residue accumulation and antibiotic resistant bacteria. It also produces a large number of adverse effects to sensitive individuals, such as sore mouth and perineal itching, dizziness, light-headedness, diarrhoea and photosensitivity. Therefore, the allowance of TC residue in foods is regulated and routinely monitored [1,2].

TC has intrinsic molecular fluorescence. The formation of a complex with lanthanide ions, e.g., Eu(III), allows intramolecular energy transfer from excited TC to the emitting level of lanthanide ions. By funneling energy to a narrow atomic emission, fluorescence intensity is enhanced by an order of magnitude. As a result, the luminescence possesses large Stokes shifts (200–300 nm) and long excitation lifetime, so both spectroscopic and temporal discriminations can be implemented to enhance selectivity. This analytical approach is known as lanthanide chelate time-resolved luminescence (TRL).

Although TRL has successfully been used for TC determination in different matrices [3–6], sample preparation is a time consuming and costly procedure. Other concerns with complicated sample preparation steps include losses of analyte, reduced precision and potential use of hazardous solvents. This poses a big obstacle to productivity especially for laboratories that routinely analyze a large number of samples [7,8]. Efforts have been devoted to simplify analytical procedure by employing improved devices or integrating

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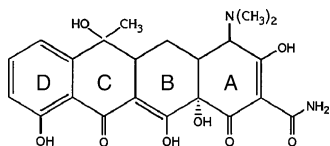


Fig. 1. Structure of tetracycline. It has been found that at neutral or lower pH, the bonding of TC with Eu(III) occurred at position 1, while at a higher pH the chelate bonding occurred at position 2 [9].

separation and spectroscopic steps into one system. For example, lanthanide ions were added to liquid chromatography systems in either a pre- or a post-column mode [9,10]. By this method, the selection of a mobile phase to obtain optimum elution should comply with the requirements of lanthanide luminescence. Paper strips with pre adsorbed europium(III) or terbium(III) were used for the simultaneous TRL determination of TC and ciprofloxacin [11] and for the determination of anesthetics, benzocaine and procaine [12]. Solid-phase microextraction was combined with room-temperature phosphorescence using Whatman 1PS phase separating paper [13] and solid-phase extraction was combined with time-resolved laser induced fluorimetry for screening polycyclic aromatic hydrocarbons [14]. Paper or cellulose membranes are most frequently used as the preferred media in solid-phase luminescence [12,13]. However, a sorbent which also can function as a spectroscopic substrate is still desired.

In this study four potential sorbents, C18, pectin, cellulose and filter paper, were examined for this purpose. TC, as a model analyte, was extracted from aqueous solutions by the sorbents and directly detected by SMTRL on the surface after coordination with europium(III) nitrate. The best candidate sorbent was selected and an analytical procedure was devised.

## 2. Materials and methods

### 2.1. Reagents and solutions

Thin-layer chromatography (TLC) plates, C18 and cellulose (all without fluorescent indicator) and all chemicals and biological reagents were purchased from Sigma-Aldrich (San Louis, MO). Tetracycline hydrochloride, europium(III) nitrate pentahydrate, disodium ethylenediaminetetraacetic acid dihydrate ( $\text{Na}_2\text{EDTA}$ ) and tris(hydroxymethyl)aminomethane (Tris) were analytical reagent grade and used without further purification. Filter paper, Whatman 42, was obtained from Fisher Scientific (Pittsburgh, PA). Deionized water (D.I. water) was prepared using a Barnstead E-pure water system (Dubuque, IA) and used to prepare all aqueous solutions.

0.10 M Tris–2.5 mM EDTA buffer solution was prepared by dissolving 12.11 g of tris(hydroxymethyl)aminomethane in 900 ml D.I. water, the solution pH was adjusted to 9.0

using 5 N HCl, 25.0 ml 0.1 M  $\text{Na}_2\text{EDTA}$  was then added and the solution was diluted to 1.0 L with D.I. water. TC stock solution was prepared in acetonitrile at 100 ppm and stored at 4 °C. TC working solution at 1 ppm was prepared daily from the TC stock solution by dilution with either Tris–EDTA buffer or D.I. water in an amber screw-capped vial. In TC enrichment experiments, dilute TC in water solutions at the concentrations of 10 or 1 ng/ml were prepared directly in 500 ml Teflon beakers from the TC working solution. Eu(III) solution, 2.5 mM  $\text{Eu}(\text{NO}_3)_3$ –2.5 mM  $\text{Na}_2\text{EDTA}$ , was prepared either in 0.1 M Tris buffer at pH 9 or in D.I. water at pH 7.

### 2.2. Sorbent preparation

The C18 TLC plate used is a thin layer of silica gel derivative backed by a glass substrate. The plate was cut into 25 mm × 8 mm strips; the silica layer was carefully cut to a 10 mm × 6 mm rectangle in the central portion. Cellulose (backed with a polyester film) and filter paper were cut into 10 mm × 6 mm rectangles and mounted on glass strips (25 mm × 8 mm) using double-sided adhesive tape.

Pectin gels were cast from a pectin solution. Pectin (degree of esterification, 93%; content of galacturonic acid, 63%; from citrus peel) solution was prepared by suspending pectin powders in D.I. water at 2% (w/v), followed by stirring at room temperature for 18 h. The pectin solution, 100  $\mu\text{l}$ , was evenly loaded on a glass strip (25 mm × 8 mm) and freeze-dried. This “loading-drying” cycle was repeated four times. The pectin layer was trimmed to 10 mm × 6 mm, rinsed with 0.5 M  $\text{CaCl}_2$  and D.I. water, 10 ml for each, and air-dried. All solid surfaces were prepared in a clean environment and stored in a desiccator at room temperature.

### 2.3. Apparatus

A commercial fluorescence spectrometer (Model Cary Eclipse, Varian, Walnut Creek, CA) was used for SMTRL measurement. The instrumental operation and signal processing were handled by Cary Eclipse Lifetime software. The sample holder shown in Fig. 2 is modified from the original cuvette holder to accommodate the sorbent strip. The vertical mounting plate has a 10 mm × 6 mm (width × height) rectangular window opening to expose the surface of substrates to

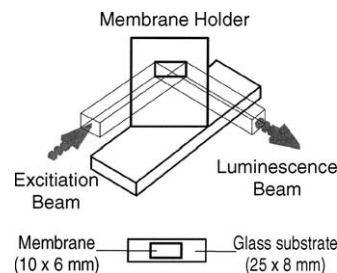


Fig. 2. Sample holder for solid-matrix time-resolved luminescence (SMTRL).

the excitation beam, which has a cross section of approximately 7 mm × 3 mm (width × height) in this position. Both the height of the window and the holder position were carefully adjusted to fully intercept the excitation beam and to maximize the signal beam intensity received by the photomultiplier tube in the detector compartment. The excitation beam incidence angle was optimized at 60°. To minimize stray light, the front surface of the mounting plate was painted flat black. The glass strip was mounted against the back of the holder plate using two binder clips, with the surface exposed to the light through the window.

#### 2.4. Scanning electron microscopy (SEM)

All solid surfaces for TC extraction and SMTRL measurement were pre-frozen at −20 °C overnight and lyophilized at −78 °C for 24 h. Dry samples were mounted on aluminum specimen stubs with double-sided adhesive tape. The glass side was facing downward while the surface side was coated with a thin layer of gold by DC sputtering. Images of topographical features were made using a model JSM 840A scanning electron microscope (JEOL USA, Peabody, MA) coupled to an Imix-1 digital image workstation (Princeton Gamma Tech, Princeton, NJ). The average pore size of solid surfaces was measured directly on the SEM micrographs and calculated as described previously [15].

#### 2.5. SMTRL for TC determination

The SMTRL measurement was performed with two different reagent-loading sequences. For one sequence, 10 µl of Eu(III) solution (pH 9) was first loaded onto the surface followed by drying over calcium sulfate in a dark desiccator at room temperature for 30 min; then spotted with 10 µl of TC working solution, and dried again. For another sequence, Eu(III) and TC were loaded in a reversed order. After sample loading, the sorbent strip was mounted on the sample holder. The SMTRL was measured at room temperature at the following setting: excitation wavelength: 388 nm, emission wavelength: 615 nm, both excitation and emission slit widths: 20 µm. For each run, ten cycles were collected and averaged. The signal intensity was integrated over 60–800 µs interval. The luminescence lifetime was calculated.

For comparison studies, the drying process was skipped in some experiments, SMTRL measurements were done on wet solid surfaces right after reagent loading.

#### 2.6. TC adsorption

For dilute TC solutions, TC enrichment was accomplished by repeated spotting or immersion with TC solution. For repeated spotting, 10.0 µl of 1.0 ng/ml TC solution was loaded onto a Eu(III) pre-absorbed solid surface and dried in a desiccator. The spotting-drying cycle was repeated 10 times prior to SMTRL measured. For the immersion approach, three pieces of Eu(III) pre-loaded sorbent strips were placed into

a Teflon beaker containing 450 ml TC solutions at 1.0 or 10.0 ng/ml. After gently shaking at 4 °C for desired time intervals, the solid sorbents were removed, rinsed with D.I. water, re-loaded with Eu(III) if necessary, and measured by SMTRL.

Alternatively, TC was desorbed from the solid surfaces by washing with methanol and the amount of desorbed TC was determined in aqueous solution by TRL [13]. Briefly, each sorbent strip was treated with 2.0 ml methanol solution (methanol:acetonitrile:D.I. water; 5/3/2, v/v) by lightly sonicating for 10 min. The solution was concentrated to 0.4 ml by placing in a desiccator connected with vacuum line, adjusted to pH 9 using Tris-EDTA, then measured by TRL. The amount of adsorbed TC ( $N_s$ ) and the amount of TC remaining in liquid phase after extraction ( $N_l$ ) were used for the calculation of the distribution constant  $K$ , according to the formula  $K = N_s V_l / V_s N_l = C_s / C_l$  [16]. Where,  $V_s$  is the volume of solid surfaces as estimated from their dimensions;  $V_l$  is the volume of liquid phase;  $C_s$  and  $C_l$  are the TC concentrations calculated for solid phase and aqueous phase, respectively.

#### 2.7. Eu(III) desorption

The Eu(III) desorption from solid surfaces was evaluated by immersing the sorbent strips in D.I. water for desired time. The amount of Eu(III) remaining on the sorbent surface was determined by SMTRL after TC loading.

### 3. Results

#### 3.1. Physical and chemical properties of solid phase surfaces

The four types of sorbents were examined for their architecture and composition in the context of TC adsorption and SMTRL measurement. Among these materials, C18, cellulose and filter paper are well known media in chromatography and have been used for TC analysis.

The micrographs of the sorbent surfaces are shown in Fig. 3. All samples were porous and the pores were interconnected. C18 and cellulose had smoother surface morphology than filter paper and pectin. C18 and cellulose had similar pore size, which was smaller than those found in pectin and filter paper. In addition, C18 and cellulose were characterized by a narrow pore size distribution. Pectin had the largest average pore size among all samples. The pores in pectin and filter paper were extremely uneven (Fig. 3, Table 1). Moreover, pectin had limited swellability upon contact with aqueous solution; the swelling behavior of other sorbents was negligible.

The four sorbents also differ from each other in chemical composition. C18 is a silica gel impregnated with hydrocarbon compounds to form nonpolar, hydrophobic zones within the gel and to coat the silica gel particles. Both cellulose and filter paper are the derivatives of natural plant polysaccha-

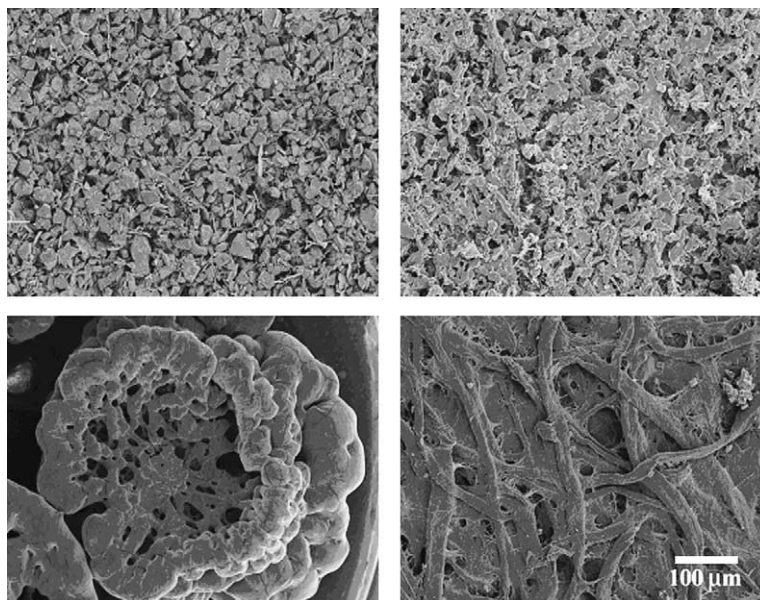


Fig. 3. SEM photographs of the four sorbents studied. Clockwise from top left: C18, cellulose, filter paper and pectin.

rides and are hydrophilic. The calcium cross-linked pectin is another polysaccharide-based sorbent consisting of both hydrophilic and hydrophobic domains. Hydroxyl groups are abundant on the surfaces of the three polysaccharide-based sorbents, while surface hydroxyl groups on the C18 are mostly blocked. The pectin also contains carboxylic groups, calcium, sodium and potassium ions.

### 3.2. SMTRL measurement

To investigate if a TC and Eu(III) complex can be formed on the sorbents, 10  $\mu$ l of Eu(III) solution, pH 9, was first loaded onto the surface of a sorbent and SMTRL measured. Some background TRL signal was observed with minor differences in intensity among the sorbents. However, a strong TRL signal at 615 nm was recorded when 10  $\mu$ l of 1.0 ng/ $\mu$ l TC solution was added to the surface following the Eu(III) loading. This phenomenon was observed for all tested sorbents. A typical example obtained on C18 is shown in Fig. 4. The results indicated the formation of TC/Eu(III) complexes on the sorbent surfaces and confirmed the intramolecular energy migration from excited state of TC donors to Eu(III).

To optimize the SMTRL measurement, luminescence of TC/Eu(III) complexes on C18 was measured under different conditions, including presence or absence of water, pH,

reagent loading sequence, and Eu(III) concentration. As shown in Fig. 4, an obvious enhancement in TRL intensity was obtained from the dry solid surface in comparison with the wet one. In another experiment, we investigated the possibility to alter the binding site of TC to Eu(III) on solid surfaces using TC solutions of two different pH values. C18 surfaces were loaded with 10  $\mu$ l of Eu(III) in D.I. water (pH 7.0), and the C18 were then divided into two groups. One was spotted with TC in Tris–EDTA buffer (pH 9) and the other with the same amount of TC in D.I. water. The SMTRL measurement showed a higher luminescence in the pH 9 group. However, the difference between the two groups was reduced by spotting an aliquot of Tris–EDTA buffer (TC-free, pH 9) to the second group (data not shown). Fig. 5 shows the luminescence signals received on C18 surfaces with different reagent loading sequences. A much higher intensity was obtained when Eu(III) solution was seeded onto the surface prior to TC loading.

Fig. 6 shows the effect of Eu(III) concentration on luminescence of TC/Eu(III). The luminescence increased with increasing concentration of Eu(III), reaching the highest intensity at 25 nmole of Eu(III). After that, increasing Eu(III) loading did not affect the luminescence. Although the mole ratio between the TC and Eu(III) in the complex is 1:1, pre-loaded Eu(III) in a large excess (as calculated, TC:Eu(III)

Table 1  
Surface properties of the four sorbents

Matrix	Thickness ( $\mu$ m)	Volume of matrix (ml)	Pore size ( $\mu$ m)	Particle size	Smoothness
C-18	250	0.015	6	5–17	Smooth
Cellulose	100	0.006	6	2–20	Smooth
Filter paper	200	0.012	>2.7	>20	Rough
Pectin	300	0.018	>50	>20	Rough

Data obtained from providers, except those related to pectin, which was measured as described in the experimental section. Volumes of solid matrices were calculated from the dimensions.

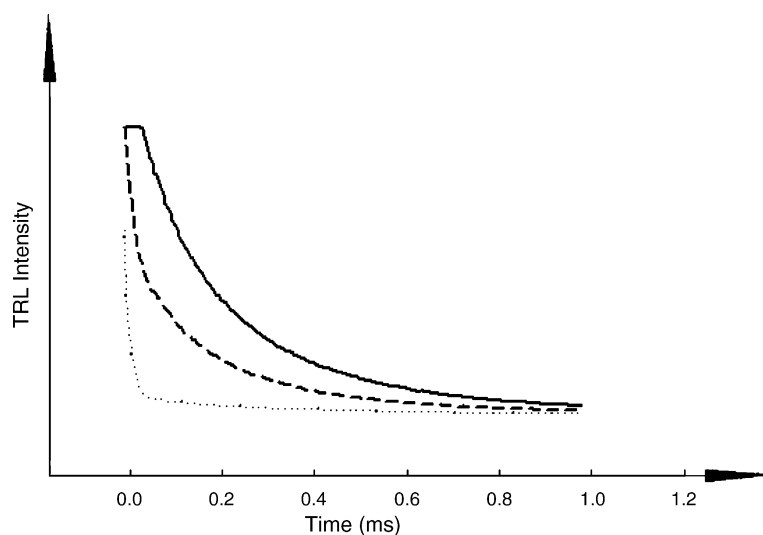


Fig. 4. Sensitivity of tetracycline detection by SMTRL method. Luminescence obtained in C18 from Eu(III) alone (dotted line) and TC/Eu(III) complex with (solid line) or without (dashed line) desiccation.

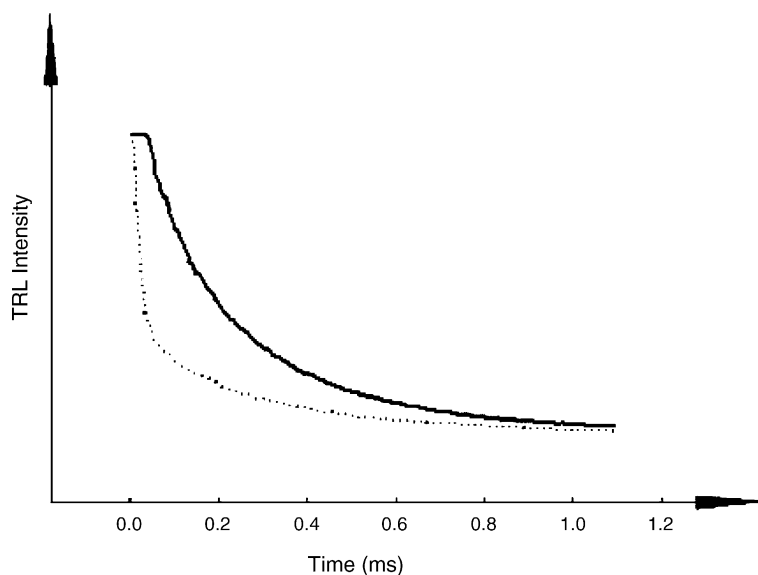


Fig. 5. Effect of reagent-loading sequence on SMTRL sensitivity detection. TC was spotted on Eu(III) pre-loaded C18 (solid line) and the opposite order (dotted line).

was 1:1, 250 when evaluated on a C18 surface) is essential to maximize SMTRL.

### 3.3. Comparison of sorbents for SMTRL and TC adsorption

As shown in Table 2, SMTRL signal intensity varied with the types of sorbents in the sequence of C18 > cellulose > pectin  $\approx$  filter paper. The luminescence lifetime of TC/Eu(III) complex on the sorbent surfaces also varied in the same sequence.

Since Eu(III) pre-loaded solid surfaces demonstrated a higher SMTRL efficiency (Fig. 5), the capability of Eu(III)

Table 2

Luminescence of europium(III)–tetracycline adsorbed on various sorbents

Sorbents	Relative intensity	Lifetime (ms)
C-18	$116.2 \pm 1.4$	>1.0
Cellulose	$53.5 \pm 10.9$	>1.0
Filter paper	$15.4 \pm 2.1$	0.8
Pectin	$19.2 \pm 2.9$	0.7

25 nmol of Eu(III) in pH=9 Tris buffer and 10 ng TC in D.I. water, were loaded into each matrix in sequence. The reagent loaded matrices were dried over drierite in a desiccator at room temperature for 30 min before SMTRL measurement. Data expressed as mean  $\pm$  S.D. ( $n=3$ ).



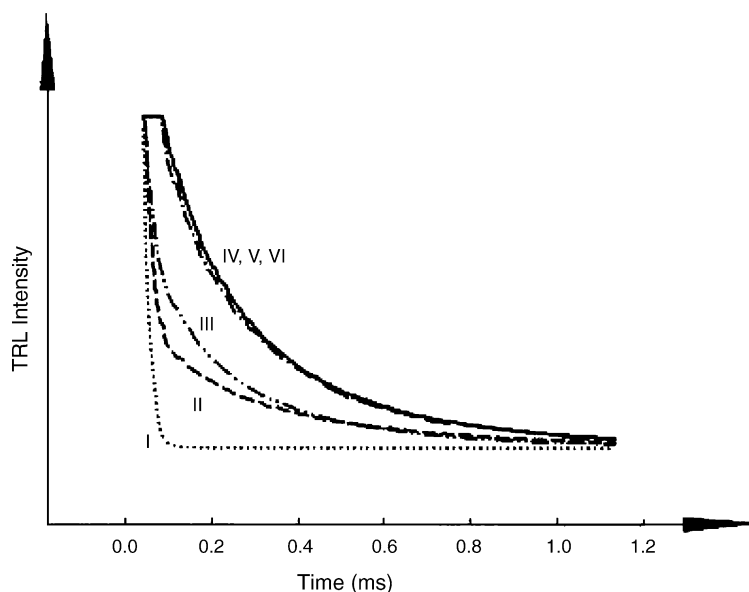


Fig. 6. Effect of Eu(III) content on TC/Eu(III) SMTRL measurement. The C18 was pre-loaded with Eu(III) of various amounts: 10 nmol (I, II), 20 nmol (I, III), 25 nmol (I, IV), 250 nmol (I, V) and 500 nmol (I, VI). 0.02 nmol TC was spotted to samples II–VI, and SMTRL measured.

pre-loaded sorbents in TC extraction was evaluated. First of all, the stability of pre-loaded Eu(III) in water had to be investigated. No desorption of Eu(III) from C18 was detected up to 12 h immersion in 150 ml D.I. water. In contrast, significant desorption of Eu(III) from other sorbents was detected after 12 h immersion (data not shown). These results demonstrated that the C18 retained Eu(III) stronger than the other three sorbents.

Fig. 7 shows the kinetics of TC extraction by C18 and cellulose. With Eu(III) pre-loaded C18, TC adsorption increased rapidly in the beginning, and reached a plateau in

12 h, indicating equilibrium was achieved. With cellulose, TC adsorption increased slowly. At equilibrium the amount of TC found on cellulose was much lower than that on C18. Table 3 shows the capability of all sorbents in TC extraction. By the immersion approach, TC was detectable on C18 at 1.0 ng/ml level, while detectable for all other solid surfaces at the 10 ng/ml level. At 10 ng/ml, the signal on C18 was about 12 times those on cellulose, filter paper or pectin. By the repeated spotting approach with  $10 \times 10 \mu\text{l}$  TC solution, TC was detectable on all sorbents even at 0.1 ng, the luminescence intensity increased in the same order of sorbents as

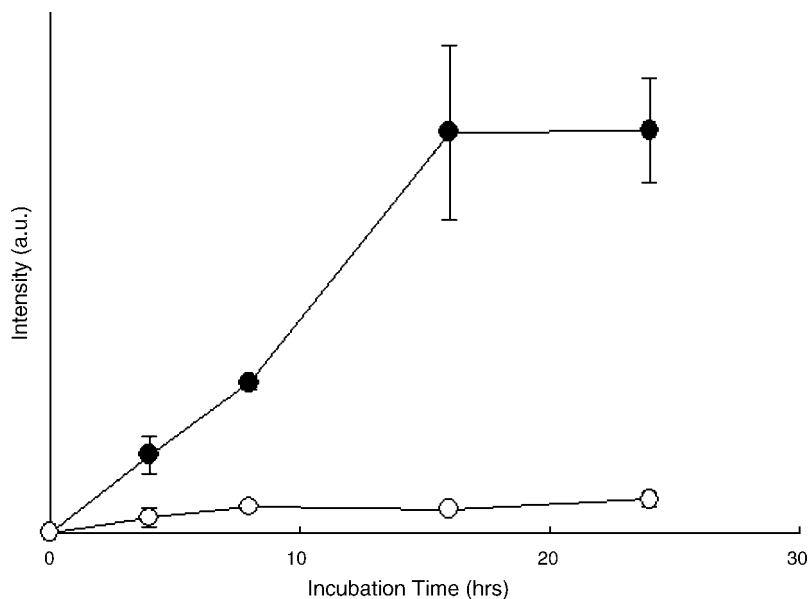


Fig. 7. Enrichment of TC from 10 ng/ml TC solution by C18 (●) and cellulose (○). Each sorbent (10 mm  $\times$  6 mm) was pre-loaded with Eu(III), immersed in 150 ml of TC solution and shaken at 4 °C for given period. At the end of experiments, the matrices were removed, rinsed with D.I. water, desiccated and analyzed by SMTRL. For cellulose, an additional 25 nmol of Eu(III) were spotted on the surface after its removal from water. All tests were in triplicate.

Table 3  
Luminescence and sensitivity of TC enriched by two loading methods

Matrix	SMTRL intensity		LOD <sup>a</sup> (ng/ml)	Linear range (ng/ml)	
	Immersion model (ng/ml)				Repeated spotting model, 10 ng
	1	10			
C-18	9.3 ± 3.6	231 ± 30.2	17.5 ± 0.4	0.7	0–1000
Cellulose	N/D <sup>b</sup>	19.2 ± 4.2	8.3 ± 2.7	5.2	0–700
Filter paper	N/D	15.3 ± 3.6	2.7 ± 0.5	–	–
Pectin	N/D	17.7 ± 3.9	2.5 ± 0.7	–	–

<sup>a</sup> The limits of detection (LOD) were calculated by  $LOD = 3S_B/m$ , where  $S_B$  is the standard deviation of the blank measurements and  $m$  is the plot slope of SMTRL intensities versus concentration. Both LOD and linear range were obtained with the solid matrices on which TC was loaded by repeated spotting method.

<sup>b</sup> N/D: not detectable.

Table 4  
Calculated distribution constants  $K$  of TC for the four sorbents

Matrix	C18	C18 with pre-loaded Eu(III)	Cellulose	Filter paper	Pectin
$K = C_s/C_l$	490	640	254	127	1270

$C_s$  and  $C_l$ : the concentrations of TC on solid matrices and liquid phase, respectively. TC on the solid matrices was desorbed by methanol washing and the amount was determined by TRL measurement in aqueous solution.

shown by the immersion method. In comparison to cellulose, a lower LOD and a larger linear range were obtained for C18.

The TC distribution between aqueous phase and extraction phase is shown in Table 4. The distribution constant is a measure of affinity of the extraction phases to the analyte. A higher  $K$  value indicates a higher affinity between the sorbent and TC. Calcium cross-linked pectin was able to extract much more TC from the solution than other solid surfaces; C18 also had strong affinity for TC adsorption; both the cellulose and filter paper were very poor in TC adsorption.

#### 4. Discussion

We have demonstrated that TC can be enriched by a sorbent and directly determined on the same sorbent by Eu(III)-sensitized TRL. Among the four sorbents tested, C18 showed beneficial properties in both sample preparation and analysis. The SMTRL measurement has several advantages over TRL conducted in solution. First of all, the SMTRL measurement only requires a small volume of sample and has high sensitivity (Table 2). Secondly, the use of solid phases minimizes the effect of water quenching on SMTRL (Fig. 4). Water molecules quench the luminescence in solution by occupying lanthanide coordination sites [17,18], therefore a synergistic co-ligand is required to diminish the effect of water quenching. With thin layer solid surfaces, adsorbed water can be removed simply by exposing the sorbents to dry air or a vacuum. The simple procedure could be attributed to the porous structure of the sorbents, which allows a quick liquid migration.

Another advantage of SMTRL is that the complex formation can be regulated directly on the sorbents. As mentioned in the results section, the addition of TC in D.I. water to Eu(III)

pre-adsorbed C18 generated low fluorescent intensity recoverable by spotting a small volume of higher pH solution on the solid surface after reagents loading. The pH dependence of SMTRL is reflected by the changes in the binding site of TC. TRL of TC and lanthanide complexes in aqueous phase is highly pH-dependent. At neutral or acidic pH, the preferential binding site in TC to Eu(III) is the enolic hydroxy group on the A ring (Fig. 1). In the solution with pH higher than 7.5, the hydroxy groups on the B, C, D rings of TC are deprotonated, thus responding to chelate bonding with the lanthanide ion (Fig. 1) [6,9,19]. The binding at B, C, D sites places the Eu(III) closer to the benzoyl moiety, resulting in an efficient energy migration and thus enhances luminescence intensity. Apparently, the binding position of TC to Eu(III) ions pre-adsorbed on the C18 can be switched from one to another by loading a small volume of aqueous solution with different pH, just as in liquid phase. It is expected that the architecture of C18 provides spaces in favor of the interaction and the liquid retained in pore areas of the solid phase facilitates the chelate bonding between TC and Eu(III).

The surface properties of solid phases, in terms of architecture and chemical composition, have an influence on TC enrichment and SMTRL. A nearly two-fold higher luminescence was observed for C18 than for cellulose with a single spot of 10 ng TC (Table 2) or 10 spots of 0.01 ng TC (Table 3). The spotting method ensures consistent reagent application to all surfaces, hence the changes in TRL intensity reflect the influence of solid surfaces on SMTRL. Both C18 and cellulose matrices are similar in physical characteristics, such as the similar pore size and particle size, and similar surface smoothness, but differ in chemical composition (Table 1, Fig. 3). It suggests that the chemical composition may play a key role in SMTRL. Likewise, pectin has the chemical structure and surface functional groups which are similar to filter paper and cellulose; the TRL intensities observed on the three surfaces resulted in the same relative order. The TRL intensities observed on filter paper and pectin were slightly lower than on cellulose; it could be attributed to the difference of their surface smoothness, as smooth surfaces tend to yield a higher signal-to-noise ratio [20].

To compare the amount of TC extracted by different sorbents with the immersion method, TC also was measured by

TRL in aqueous solution after desorption. This avoided the effect of surface-to-surface variation. The sorbent-dependent adsorption activity revealed by TRL measurements (Table 4) is different from that by SMTRL analysis (Table 3), indicating the effect of the surface properties of sorbents on TC/Eu(III) luminescence. For those sorbents with higher *K* value and lower SMTRL intensity, they may have a lower TC/Eu(III) population or a strong environmental quenching might occur [20,21]. Accordingly, the calcium cross-linked pectin is excellent in TC extraction, but not suitable for SMTRL application. Cellulose and filter paper are not the selection for both TC enrichment and SMTRL. Although the influence of solid surface on luminescence could be directly or indirectly due to surface functionalities, sugar components, impurities or physical properties, more detailed research is needed.

The interference of surface properties of a sorbent to TC/Eu(III) luminescence could be minimized by pre-loading Eu(III) ions in excess to the surface. As shown in Fig. 5, a much higher intensity was obtained on Eu(III) pre-loaded C18 than from plain C18. As mentioned above, at pH of about 9 the chelate bonding of TC occurs at positions near the benzoyl moiety, which also is responsible for TC binding to the hydrophobic C18. Supposedly, the adsorption of TC on C18 may reduce the accessibility of B, C, D rings to Eu(III) chelation. As a result, the efficiency of SMTRL is reduced. This was compensated by seeding a large amount of Eu(III) on the C18 surface. The TC molecules that were added after Eu(III) loading are most likely to be captured by the pre-adsorbed Eu(III) ions rather than landing on the bare C18 surface, resulting in a higher TC/Eu(III) complex population on the surfaces, and therefore a higher SMTRL signal. Further evidence for this mechanism was obtained by increasing the amount of pre-loaded Eu(III) on the sorbent surface (Fig. 7). The pre-loading of Eu(III) to C18 may have created a pseudo surface, which facilitated the chelate bonding of TC to Eu(III) and the luminescence.

## 5. Conclusions

We have found that TC could be measured on the surface of a C18 TLC plate by the SMTRL method. The pre-adsorption of Eu(III) on the C18 facilitated TC/Eu(III) complex formation and the subsequent luminescence. The C18 also showed a high affinity in TC adsorption. Thus, TC analysis could be simplified by the hyphenation of TC extraction and TC measurement using a single C18. This method can be applied for routine screening TC and other luminescent lanthanide-chelating analytes in environmental or biological conditions. In comparison with C18, cellulose and filter paper are poor in TC extraction and TC/Eu(III) luminescence; calcium cross-linked pectin facilitates TC adsorption but interferes with the SMTRL luminescence. The architecture and composition of C18 seem to be responsible for its distinct advantages in TC

analysis. The definite surface properties such as chemical inactivity, relative hydrophobicity and smoothness, as well as an appropriate porosity are considered as typical features of the optimal solid phase, which can fulfill the dual purposes as a sorbent for analyte extraction and a substrate for analyte detection by SMTRL. A procedure to use C18 sorbent strip for TC determination in milk has been successfully developed based on the method described in this study [22].

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